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Life-History Variation in a Colonial Ascidian: Broad-Sense Heritabilities and Tradeoffs in Allocation to Asexual Growth and Male and Female Reproduction

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Abstract. Intraspecific variation in life-history strategy provides a valuable opportunity for examining how natural selection acts on life-history variants to mold reproductive strategies. Evaluating the consequences of selection requires knowledge of the range of phenotypic variation in life histories, the extent to which variation is genetically based, and possible correlations among different traits that might constrain or promote the effect of selection on individual traits. We explored life-history variation in the colonial ascidian *Botryllus schlosseri* (a cyclical hermaphrodite) by growing clonal replicates of 18 genotypes in a common-garden experiment. Colonies of this species have previously been shown to vary in egg production and growth rate. We demonstrate that genotypes also vary in sperm production, which is manifested as variation in testis size. We then calculate broad-sense heritabilities for a suite of life-history traits and demonstrate correlations among traits that suggest a three-way tradeoff in resource allocation to asexual growth and sexual reproduction *via* male and female function. This correlation structure suggests that selection cannot act independently on individual life-history traits.

Introduction

Marine habitats tend to subject organisms to unique selective pressures that are reflected in the life-history strategies that evolve in this environment (Hendler, 1975; Strathmann *et al.*, 1984; McEdward and Coulter, 1987; Emlet *et al.*, 1987; Strathmann, 1990). For free-spawning marine invertebrates, fertilization *via* the release of sperm into the water column may lead to distinctive selective pressures on life-history strategies (Ghiselin, 1987; Strathmann, 1990). In particular, recent field experiments and assays of fertilization success in nature have suggested that selection *via* fertilization processes may profoundly influence both the quantity of gametes produced and specific attributes of those gametes (reviewed in Levitan, 1995; Levitan and Petersen, 1995).

Because many species exhibit little apparent intraspecific variation in life-history strategy, comparative studies among taxa have contributed immensely to the study of the evolution of marine invertebrate life histories (*e.g.*, Menge, 1975; Strathmann and Strathmann, 1982; Eckelbarger and Watling, 1995). However, selection actually acts on variation within a species, and some marine species do exhibit substantial intraspecific variation in different life-history characters (*e.g.*, Hughes and Hughes, 1986; Grosberg, 1988; Yund, 1991; Cohen and Strathmann, 1996). These variable taxa yield particularly valuable opportunities for evaluating the action of natural selection on life-history variants.

Evaluating the consequences of natural selection on intraspecific variation in life-history traits requires three types of information: first, which life history traits exhibit phenotypic variation, and hence can potentially be subject to selection; second, for each variable trait, what is the extent to which phenotypic variation is genetic; third, since selection ultimately acts on the total phenotype and not just on independent traits, are there positive and negative correlations among traits. Correlations among traits could either constrain or promote the effect of selection acting on each individual trait (Lande and Arnold, 1983).

We present data from a common-garden experiment with the colonial ascidian *Botryllus schlosseri*, a cyclical

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hermaphrodite, that addresses these three points. Colonies of this species are known to vary in growth rate, terminal size, and egg production (Grosberg, 1982, 1988; Chadwick-Furman and Weissman, 1995). We demonstrate additional variation among genotypes in sperm production and reproductive cycle duration, and estimate broad-sense heritabilites for all five of these traits. Finally, we explore possible correlations among traits to assess the potential for selection to act independently on individual traits.

Materials and Methods

Study species

Botryllus schlosseri, a colonial ascidian with a cosmopolitan distribution, is common on firm substrata in the shallow subtidal zone of New England waters (Gosner, 1971). Colonies are composed of asexually produced zooids arranged in clusters, or systems, with all zooids in a system sharing a common exhalant siphon. All zooids in the colony synchronously undergo an asexual cycle of zooid replacement in which a new generation of zooids (buds) forms between the existing generation of zooids (Milkman, 1967). Over a period of about 6–12 d, these buds grow and expand, and then take over the function of the previous generation of zooids, which are quickly resorbed. Colonies grow as long as bud production exceeds the number of zooids in the current generation, and growth continues until colonies reach a terminal size (generally associated with the onset of sexual reproduction), which is then maintained over a number of subsequent asexual cycles (Boyd *et al.*, 1986; Grosberg, 1988).

Sexual reproduction begins when colonies exceed a minimum size (Harvell and Grosberg, 1988). Eggs brooded by each new generation of zooids are fertilized at the time of take-over, when the zooids' siphons first open and admit water to the atrial chambers. Sperm release does not begin until about 1–2 d after ovulation, effectively preventing self-fertilization (Milkman, 1967; Yund and McCartney, 1994). Because sexual reproduction is linked to the asexual zooid replacement cycle, colonies cycle between male and female function.

Relationship between testis cross-sectional area and sperm count

When colonies are grown in culture on glass substrata, variation in sperm production is readily apparent as variation in testis size (viewed from the underside; pers. obs.). To facilitate monitoring a number of colonies and to permit repeated sampling of individuals over time, we needed an assay of sperm production that was relatively quick and nondestructive. Consequently, we assayed sperm production as testis cross-sectional area. To validate this approach, we first tested for a correlation between testis cross-sectional area and the number of sperm in individual testes. We used an ocular micrometer to measure the length and width of 27 mulberry-shaped testes in zooids of different colonies and then surgically removed each measured testis from its zooid. All testes were sampled at the time of maximum size during the reproductive cycle (day 2–3 by the criteria of Milkman, 1967). Each testis was removed by making a longitudinal incision in the wall of the zooid, lifting the testis with forceps, and cutting the connective tissue attached to the underside of the testis. Excised testes were preserved in 2% glutaraldehyde in buffered seawater and later macerated in 100 μl of seawater by five gentle grinds with a glass rod. The number of mature spermatozoa in each testis was estimated as the mean of four hemocytometer counts of the sperm suspension. We analyzed these data by examining the correlation between mean sperm count and testis cross-sectional area (calculated as the length multiplied by the width of each testis).

Common-garden experiment

We examined life-history variation among 18 *B. schlosseri* genotypes by growing clonal replicates in a flowing seawater system at the University of Maine's Darling Marine Center. Colonies used in this experiment were initially collected from widely spaced ($\approx$10–20 m. apart) locations within a field population located in 3 to 8 m of water (MLW) on the western shore of Carlisle Island in the Damariscotta River, Maine (adjacent to the experimental field site of Yund and McCartney, 1994; Yund, 1995; Atkinson and Yund, 1996). Field-collected colonies were subdivided and explanted onto glass microscope slides (2.5 $\times$ 7.6 cm), with between 5 and 11 replicate colonies established for each genotype.

The 18 genotypes selected for inclusion in the common-garden experiment were chosen on the basis of initially possessing less than three eggs per bud. Although this criterion restricted us to exploring only a subset of the total life-history variation in this population, this limitation was necessary in order for us to collect data on each colony over a series of asexual or sexual cycles. Genotypes with higher egg production were present (although rare) in this population, but such colonies typically die after one brooding cycle (Grosberg, 1988).

The experiment began on 20 June 1994, when all colonies were trimmed to an average of 33.5 zooids ($\pm$1.9 SE) arranged in two or three systems of zooids. Colonies were housed in a vertical position in acrylic racks, with neighboring slides separated by 1.3 cm. The racks were placed in a single large, shallow tank (130 $\times$ 100 $\times$ 9 cm) in the flowing seawater system. Racks had no fixed position within the tank, but systematic posi-

tional effects are unlikely because racks were moved and relocated daily throughout the experiment. Similarly, colonies were shuffled among positions within racks about once a week (at the time of each data collection). No supplemental food was added, because past experience indicated that colonies have reasonably high growth and fecundity under these culture conditions.

Data on colony size (number of zooids), growth rate (number of buds), egg production (number of eggs per bud for a subsample of 10 buds), and sperm production (testis cross-sectional area for a subsample of 10 testes in 5 zooids) were collected for each surviving colony (some replicates died before the end of the experiment) once during each asexual or sexual cycle for seven cycles, beginning the week of 27 June. Colonies were viewed from either the top (colony size) or bottom (all other variables) under a dissecting microscope. Testis cross-sectional area was calculated by measuring the length and width of testes with an ocular micrometer at 25 × magnification. Testes measurements were standardized within each asexual or sexual cycle by sampling within the window of maximum testis size (day 2–3 by the criteria of Milkman, 1967). Testes measurements and bud counts were performed on 5 and 10 zooids (respectively) widely scattered throughout the colony to prevent possible biases from positional effects within colonies. For a subset of eight genotypes, we also directly assayed variation in sperm production by excising three testes from each of three replicate colonies in cycle 6 and estimating the number of mature sperm in each testis as the mean of four hemocytometer counts of a 100-μl dilution of the macerated testis (as described in the preceding section). For each colony, we also recorded the time (number of days) required to complete each asexual and sexual cycle. Comparison of these values permits an assessment of variation in cycle duration among genotypes.

Life-history data were analyzed through a series of ANOVAs performed with a computer software package (JMP, SAS Institute, Cary, NC). Unequal sample sizes among genotypes and sample dates due to the death of some replicate colonies and values missed during data collection necessitated an unbalanced design. Similar analyses were conducted for six dependent variables: eggs per bud, buds per zooid (calculated by dividing the number of buds by the number of zooids in each colony in each cycle), testis cross-sectional area, sperm count in cycle 6, terminal size (the maximum number of zooids observed by each colony), and cycle duration. All analyses included the random effects 'genotype' and 'replicate nested within genotype', except for the analysis of terminal size which did not include a 'replicate' effect because there was only a single size value for each replicate colony. Analyses for the four dependent variables eggs per bud, buds per zooid, testis cross-sectional area, and cycle duration included 'cycle' as an effect and were performed as repeated measures ANOVAs by testing 'cycle' with respect to the random effects. The 'genotype' by 'cycle' interaction effect was omitted from these models due to inadequate sample sizes in some cells. Data collection for the two dependent variables terminal size and sperm count during cycle 6 was restricted to a single cycle, and hence no 'cycle' effect could be included in these models.

Variance components from these analyses were used to calculate the clonal repeatability of each life-history trait (a form of broad-sense heritability; Falconer, 1981). The component of variance attributable to the 'genotype' effect was used as an estimate of genetic variance and was divided by the sum of all variance components, which provides an estimate of total phenotypic variance (Falconer, 1981). We also recorded the range of genotype means and least square means (which adjust for a 'cycle' effect in four of the six analyses) for each life-history trait, and calculated the magnitude of variation in each trait by dividing the highest genotype least square mean by the lowest. Finally, we explored possible univariate and multivariate correlations among life-history traits. First, we calculated Pearson product-moment correlations among genotype least square means for each possible set of paired traits. For a subset of three of the traits (eggs per bud, testis area, and buds per zooid) we also calculated partial correlation coefficients and employed a principal components analysis to characterize the dimensionality of the three-way relationship.

Results

Relationship between testis cross-sectional area and sperm count

Testis cross-sectional area, calculated as testis length multiplied by width, was highly correlated with the actual number of mature spermatozoa in each testis (Fig. 1; $r = 0.71$, $P < 0.001$). Testes are three-dimensional objects, and so variation in cross-sectional area alone (a two-dimensional measurement) might be expected to scale nonlinearly with actual sperm counts. However, there is no evidence of a nonlinear relationship between these variables (Fig. 1). Nonlinear functions fit to these data yielded substantially lower correlation coefficients than the linear function (exponential, $r = 0.52$; power, $r = 0.48$; logarithmic, $r = 0.62$). Either variation in testis thickness (the third, unmeasured dimension) is minimal, or the effect of this aspect of variation in testis volume on sperm counts is negligible within the size range measured in this study.

Genotypic variation and clonal repeatabilities of life-history traits

All six life history traits assayed in this study varied greatly among genotypes (Table I). The effect of geno-

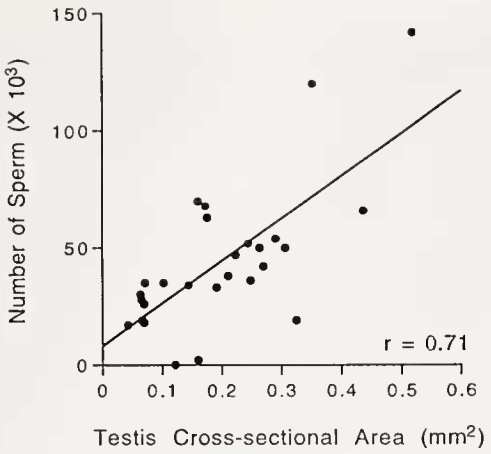

Figure 1. The relationship between testis cross-sectional area and number of mature spermatozoa within 27 testis measured and excised from different colonies.

type was extremely significant ($P < 0.0001$) in all six analyses. In contrast, the effect of replicate nested within genotype (an indicator of environmental variance) was not significant in four out of the five analyses in which it was included (Table I). Testis cross-sectional area was the only trait that exhibited significant variation among replicate colonies within a genotype, though the magnitude of the replicate effect was only about half that of the genotype effect (Table I). In all four analyses for which data were collected in multiple asexual and sexual cycles, the effect of cycle was also highly significant (Table I). Averaged across all genotypes, the number of eggs per bud and testis cross-sectional area both initially increased over the first two reproductive cycles and then declined in the final two cycles (Table II). The number of buds per zooid decreased over subsequent cycles as colonies approached a terminal size (Table II). Cycle duration fluctuated over subsequent cycles with no apparent pattern (Table II).

The temporal patterns of life-history traits can be better appreciated by examining the performance of three sample genotypes, chosen to represent the extremes of life-history strategy present among experimental colonies (Fig. 2). All three genotypes exhibited high initial growth rates and then leveled off at a maximum size (Fig. 2; number of zooids), but the high-growth genotype (Fig. 2C) grew more rapidly than the other two (Fig. 2A, B) and never completely ceased growing. Egg production generally increased over subsequent cycles, but substantial variation was present between adjacent cycles (Fig. 2; eggs per bud). Finally, though sperm production generally increased over subsequent cycles (Table II), sperm production in the high-sperm-production genotype actually decreased over time (Fig. 2A; testis area), a pattern that was repeated for the second highest sperm-production genotype in this study (unpubl. data). All of the lower sperm-production genotypes either increased sperm production over time or remained more-or-less constant (Fig. 2B, C).

Clonal repeatabilities calculated from variance components from these analyses varied widely among traits (Table III). The two variables with the highest expected environmental component to variance (buds per zooid and cycle duration) had the lowest clonal repeatabilities (Table III). The relatively high clonal repeatability for actual sperm counts may be due to a combination of sampling in a single cycle, limiting data collection to only eight genotypes, and utilizing a higher resolution sampling technique (in comparison to testis cross-sectional area), all of which could have reduced environmental variance.

Although all six life-history traits varied significantly among genotypes (Table I), the magnitude of that variation differed greatly among traits (Table III). Cycle duration exhibited the least variation, with the least square mean for the slowest genotype only 40% greater than that

Table I

ANOVA results for the six life-history variables

Source	df	Sum of squares	*F*	*P*
A. Dependent: Eggs per bud				
Genotype	17	199.1	38.9	0.0001
Replicate [Genotype]	123	34.4	0.7	0.9979
Cycle	6	78.4	30.4	0.0001
B. Dependent: Testis cross-sectional area				
Genotype	17	21.8	11.4	0.0001
Replicate [Genotype]	93	11.3	1.7	0.0005
Cycle	6	8.6	19.9	0.0001
C. Dependent: Sperm count in cycle six				
Genotype	7	183,698	28.7	0.0001
Replicate [Genotype]	16	14,609	1.0	0.4781
D. Dependent: Buds per zooid				
Genotype	17	16.7	9.3	0.0001
Replicate [Genotype]	107	10.6	0.7	0.9958
Cycle	6	115.1	150.8	0.0001
E. Dependent: Terminal colony size (number of zooids)				
Genotype	17	39,101	9.0	0.0001
F. Dependent: Cycle duration				
Genotype	17	116.6	13.0	0.0001
Replicate [Genotype]	106	44.7	0.3	0.9999
Cycle	5	136.1	22.1	0.0001

Note: All analyses contain genotype as a random main effect. Analyses for all dependent variables except for terminal colony size contain replicate nested within genotype as a second random main effect. Analyses for all dependents except for terminal colony size and sperm count in cycle 6 contain cycle as an additional main effect. These four analyses were performed as repeated measures by testing the cycle effect with respect to the random effects.

Table II

Cycle effects for the four life-history variables measured over subsequent asexual and sexual cycles

Cycle number	Eggs per bud	Testis area	Buds per zooid	Cycle duration
1	1.12	0.64	2.25	—
2	1.63	0.99	1.85	6.74
3	1.61	0.78	1.11	8.10
4	2.01	0.79	1.01	7.22
5	2.21	0.99	1.08	7.06
6	1.57	0.80	0.96	7.78
7	1.34	0.58		8.17

Note: Least square means, adjusted for genotype and replicate nested within genotype effects, are reported for each cycle. Cycle duration values are missing for the first cycle because the start of the cycle preceded the initiation of data collection. Similarly, buds per zooid data are missing for cycle seven because the number of zooids was not counted in what would have been the eighth cycle.

of the fastest genotype (Table III). Thus, although we were able to detect significant variation in cycle duration among genotypes, the magnitude of variation in this trait relative to other life-history traits is comparatively minor. The other five variables exhibited variation in genotype least square means ranging from more than a factor of 2 (buds per zooid) to almost a factor of 5 (sperm count in cycle six; Table III).

Correlations among life-history variables—genotype means

Two pairs of correlations among the six life-history traits that we measured exist because the pairs of variables measure traits produced by the same or very similar underlaying processes. First, for the subset of eight genotypes for which we actually counted sperm in cycle 6, this variable was highly correlated with genotype least square means for testis cross-sectional area (Table IVA). Because the two variables just represent two different ways of measuring sperm production, this correlation is to be expected and conveys little biological information (beyond confirming the relationship between testis size and sperm number; see Fig. 1). Secondly, genotype least square means for growth rate (buds per zooid) were significantly correlated with genotype means for terminal size (Table IVA). Again, genotypes with higher growth rates (adjusted for variation among cycles) should in general attain a larger terminal size. These two correlations are assumed to be biologically relatively trivial, and the remainder of our presentation considers only one of each of these two pairs of variables (genotype least square means for testis cross-sectional area and buds per zooid, since these two traits are measured at the zooid level and adjusted for variation among cycles).

Of the remaining four life-history variables, genotype least square means for cycle duration were not significantly correlated with genotype least square means for any of the other variables (Table IVA). Since the magnitude of variation in cycle duration was so much smaller than for the other three variables (Table III), correlations might exist that could be detected only with much larger sample sizes. Alternatively, variation in cycle duration may be truly independent of other life-history traits.

The genotype least square means of the three remaining variables, which represent allocation to female repro-

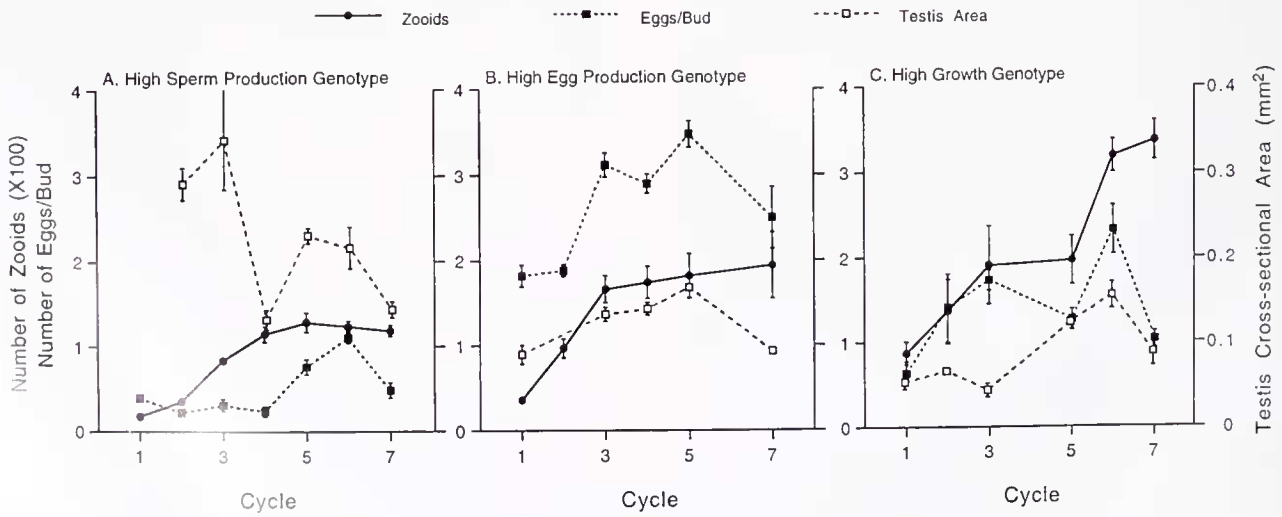

Figure 2. Growth and sexual reproduction trajectories for three of the more extreme *Botryllus schlosseri* genotypes in this study. Average eggs per bud, colony size, and testis cross-sectional area are plotted against cycle number. Error bars represent 1 standard error. (A) A high-sperm-production genotype. (B) A high-egg-production genotype. (C) A high-growth genotype.

Table III

Clonal repeatabilities, genotype ranges, genotype least square means ranges (adjusting for cycle effects in four of the variables), and the magnitude of genotype least square means variation (highest genotype value divided by lowest genotype value) for the six life-history variables

Variable	Clonal repeatability	Genotype means		Genotype least square means		
		Highest	Lowest	Highest	Lowest	Magnitude
Eggs per bud	0.47	2.63	0.68	2.77	0.62	×4.5
Testis area (mm^2)	0.42	0.23	0.08	0.25	0.07	×3.5
Sperm count ($\times 10^3$)	0.75	196.4	42.0	196.4	42.0	×4.7
Buds per zooid	0.21	1.89	0.99	1.92	0.79	×2.4
Terminal size	0.57	338.3	78.3	338.3	78.3	×4.3
Cycle duration	0.20	8.19	6.33	8.68	6.28	×1.4

duction (eggs per bud), male reproduction (testis cross-sectional area), and asexual growth (buds per zooid) at the zooid level, exhibit somewhat more complex correlations. First, there are significant negative univariate correlations between buds per zooid and both eggs per bud and testis area (Table IVA). In contrast, the correlation between eggs per bud and testis area is virtually zero (Table IVA). However, the partial correlation coefficients between each pair of traits (which in each case adjust for the effect of the third variable) suggest a much stronger relationship in three-dimensional space. All three partial correlations are much more strongly negative than the respective univariate correlations, especially the partial correlation between eggs per bud and testis area (Table IVB). This pattern suggests a negative, three-way correlation among these three variables that is consistent with a tradeoff in resource allocation among male, female, and asexual reproduction.

These negative correlations can also be examined by visualizing the relationships among these variables in three dimensions (Fig. 3). The data points fall primarily on a plane that intersects the egg per bud, testis cross-sectional area, and buds per zooid axes at relatively high values (Fig. 3). A principal components analysis provides an additional assessment of the dimensionality of this data cloud (Table V). The first two principal components have relatively high eigenvalues and in combination explain more than 92% of the variance in the data set (Table V). The third principal component has a low eigenvalue and explains less than 8% of the variance (Table V). This result indicates that the data points cluster

Table IV

Correlations among genotype least square means

	Testis area	Sperm count	Buds per zooid	Terminal size	Cycle duration
A. Univariate (Pearson's product moment) correlation coefficients between each pair of variables (all pairs)[1]					
Eggs per bud	0.01 NS	−0.21 NS	−0.59**	−0.34 NS	−0.06 NS
Testis area		−0.87**	−0.48*	−0.46 NS	0.12 NS
Sperm count			−0.41 NS	−0.30 NS	0.02 NS
Buds per zooid				0.52*	0.19 NS
Terminal size					−0.09 NS
B. Partial correlation coefficients among the three zooid-level variables[2]					
Eggs per bud	−0.48*		−0.67**		
Testis area			−0.59**		

Note: $*P < 0.05$; $**P < 0.01$; NS = Not Significant.

[1] $n = 18$ genotypes in all comparisons (16 df) except those involving sperm count in cycle 6 for which $n = 8$ (6 df).

[2] $n = 18$ in each comparison (15 df). These values represent the correlations between each pair of variables after adjusting for the effect of the third variable.

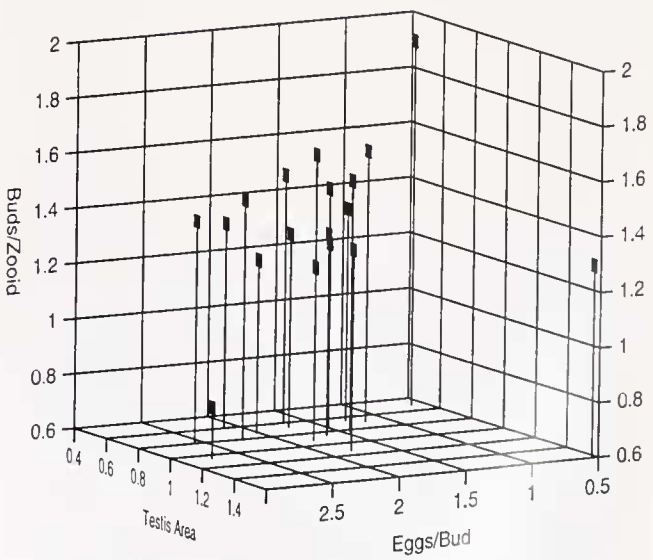

Figure 3. The three-dimensional relationship among genotype least square means for eggs per bud, testis cross-sectional area, and buds per zooid. Values for the 18 genotypes fall near a plane that slopes downward from the upper back to the lower front of the figure.

Table V

Principal components analysis of the three-way relationship among eggs per bud, testis cross-sectional area, and buds per zooid

	Principal components		
	PC 1	PC 2	PC 3
Eigenvalue	1.77	0.99	0.24
Percent of variance	59.00	33.04	7.96
Cumulative percent of variance	59.00	92.04	100.00
Eigenvector loadings			
Eggs per bud	0.55	−0.63	0.55
Testis area	0.45	0.78	0.44
Buds per zooid	−0.71	0.00	0.71

Note. The first two principal components explain most of the variance in the data and are heavily loaded on the three variables, indicating that the data points fall largely on a plane that represents a three-way tradeoff in allocation to male, female, and asexual reproduction (see text for details). Because there are only three variables in the analysis, the third principal component is completely determined by the first two; it has to explain all of the remaining variance. However, the very low magnitude of the third component is not inevitable, and it is the relative magnitude of the three principal components that describes the planar nature of the relationship among the three primary variables.

mainly on and around a plane defined by the first two principal components. The first principal component is most heavily loaded (negatively) on the variable buds per zooid, whereas the second principle component is heavily loaded on eggs per bud (negatively) and testis cross-sectional area (positively; Table V). Again, the principal components analysis demonstrates a three-way negative correlation among traits associated with male, female, and asexual reproduction.

Discussion

Genetic and environmental variation

As previously reported for other populations (Grosberg, 1988; Chadwick-Furman and Weissman, 1995), *B. schlosseri* colonies in the Damariscotta River exhibited a great deal of variation in egg production and growth rate (Table III). In addition, we found colonies to be highly variable in sperm production, with the highest sperm-production genotype yielding 4.7 times as many sperm per testis as the lowest sperm-production genotype (Table III). Variation in sperm production is reflected in the size of testes, and measurement of testis cross-sectional area provides a reasonable nondestructive [illegible] of actual sperm production (Fig. 1).

In contrast to a previous study (Grosberg, 1982, 1988), we did not detect significant variation in reproductive cycle duration among genotypes. Since a colony's growth rate in reality is a function of both bud production per zooid and cycle duration, the existence of genotype-specific cycle duration suggests that caution must be used in comparing colony growth rates on the basis of budding rates alone. However, the magnitude of variation in cycle duration is relatively small compared to variation in bud production (Table III), and cycle duration does not appear to be correlated with other life-history traits. Consequently, for many purposes the inclusion of information on cycle duration may only increase variance in growth rates.

To assess temporal patterns in four of the life-history variables, we were forced to exclude genotypes with very high egg production from our common-garden experiment. Colonies with higher levels of egg production than those included here typically exhibit semelparous reproduction and then die (Grosberg, 1988), precluding the collection of data on subsequent asexual and sexual cycles. Consequently, our value for the magnitude of variation in egg production (Table III) somewhat underestimates the total variation in this natural population. Qualitatively, however, total variation in egg production in the Damariscotta River still appears to be quite a bit lower than in the Eel Pond at Woods Hole (Grosberg, 1988). Although we have collected colonies that produced up to 6 eggs per bud, colonies with egg production > 3 eggs per bud appear to be rare at all times of the year (unpubl. data). In contrast, colonies producing 8 to 12 eggs per bud dominate populations in the Eel Pond in early summer (Grosberg, 1988). Although we know that we underestimated total phenotypic variation in egg production, we can only speculate on the effect that excluding higher egg producers may have had on our estimates of variation in other life-history traits. If the negative correlations among male, female, and asexual reproduction (Fig. 3, Tables IV and V) are maintained across higher levels of egg production, then the range of variation in the other life-history traits may have been affected as well. Consequently, our range estimates for life-history traits (Table III), though demonstrating a large degree of variation in this population, are likely to be conservative. However, the correlation structure itself may be altered if more extreme genotypes are included in the analysis (Grosberg, 1988).

Following colonies through subsequent asexual and sexual cycles allowed us to evaluate temporal trends in female (eggs per bud), male (testis cross-sectional area), and asexual (buds per zooid) reproduction as well as cycle duration (Table II). All colonies were sexually mature when collected from the field, so the temporal patterns reported here do not simply reflect the onset of reproduction. In particular, significant variation in egg and sperm production among subsequent cycles suggests that estimates of reproductive output based on observations of a single cycle, even of sexually mature colonies, should be interpreted with caution. Additionally, the different tem-

poral patterns exhibited by different genotypes (Fig. 2) are suggestive of genotype by cycle interaction effects, which we could not explicitly test because of inadequate sample sizes in some cells. The temporal component of variation in life-history strategy in *B. schlosseri* merits a more detailed examination.

All six life-history variables that we measured exhibited significant variation among genotypes (Table I), with clonal repeatabilities (a form of broad-sense heritability) ranging from 0.20 to 0.75 (Table III). These broad-sense heritability estimates may include some forms of environmental variance, and so set an upper limit for the proportion of additive genetic variance (Falconer, 1981). Although the relatively large magnitude of most of our broad-sense heritability estimates suggests a large genetic component to phenotypic variation, environmental effects are also likely to be substantial. Grosberg (1988) has previously demonstrated that food levels can significantly alter both asexual growth rates and egg production levels in *B. schlosseri*. The temporal patterns that we observed in egg and sperm production among reproductive cycles (Table II) may reflect temporal variation in planktonic food availability. In particular, the decrease in reproductive output during the last two cycles coincided with a previously reported seasonal decrease in phytoplankton in the Damariscotta River (Ineze *et al.*, 1980). Likewise, temporal patterns in cycle duration (Table II) may be due to variation in water temperature (Grosberg, 1982). Clearly, the results reported here do not constitute a definitive statement about the genetic basis of these life-history traits. Breeding experiments will ultimately be necessary to estimate narrow-sense heritabilities.

The terminal sizes of colonies in our study were substantially smaller than those reported in other studies that did not employ clonal replication (Grosberg, 1988; Chadwick-Furman and Weissman, 1995; etc.). Either colonies in the Damariscotta River cease growth at a smaller size than colonies in some other populations (Monterey Bay and the Eel Pond), or our estimates of terminal size were affected by subdividing colonies to produce clonal replicates. Field colonies in the Damariscotta River appear to frequently attain a larger size than those in our study (pers. obs.), lending credence to the latter interpretation. Although subdivision may have affected our absolute values for terminal size, all genotypes were subdivided, and hence the terminal size of subdivided colonies should nevertheless yield a valid estimate of the relative performance of each genotype.

Correlations and selection

The six life-history variables that we measured were not independent of one another. In addition to a couple of correlations between variables that measure the same or similar traits (testis cross-sectional area and sperm counts, growth rate and terminal size), we found negative partial correlations among genotype least square means for male (testis cross-sectional area), female (eggs per bud), and asexual (buds per zooid) reproduction (Table IV). The three-dimensional relationship of these variables (Fig. 3) and the results of a principal component analysis (Table V) both suggest a three-way tradeoff among these variables. Although this three-dimensional relationship is greatly strengthened by the inclusion of the three most extreme genotypes, the remaining 15 genotypes still cluster around a plane (Fig. 3) and display the same basic relationship.

One element of this multivariate correlation appears to differ slightly from the result previously reported by Grosberg (1982), who found no correlation within iteroparous colonies between asexual growth (buds per zooid) and female reproduction assayed as eggs per bud (male reproduction was not assayed). However, Grosberg (1982) did detect a negative correlation between asexual growth and lifetime egg production, which is another assay of female reproduction. Strikingly, the correlation between asexual growth and female reproduction (assayed as eggs per bud) became strongly positive when semelparous genotypes were also included in the analysis (Grosberg, 1982, 1988).

The existence of negative correlations alone indicates little about the proximate causes of life-history tradeoffs. Surgical manipulation of gamete production and growth patterns (Grosberg, 1988) could be employed to explore possible physiological mechanisms underlaying these negative correlations. However, the correlation structure that we detected is consistent with a simple energetic tradeoff in allocation to male, female, and asexual reproduction. Although a tradeoff in allocation between male and female reproduction is a common assumption of sex-allocation models for hermaphrodites (Charnov, 1979, 1982), this assumption has rarely been empirically evaluated. Although the likely consequence of a shift in resource allocation between male and female reproduction is relatively straightforward, variation in allocation to asexual reproduction has potentially more complex ramifications. Asexual reproduction increases the number of zooids in a colony and hence is likely to fundamentally alter the future energy budget of a colony by determining total food intake. Could increased allocation to asexual growth at the expense of current sexual reproduction be associated with increased sexual reproduction effort at some later point in colony ontogeny? Again, temporal allocation patterns of different genotypes (genotype by cycle interactions) merit further consideration.

The negative correlations among male, female, and asexual reproduction that we detected have important

implications for evaluating the possible consequences of selection on these life-history traits. These negative correlations are based on genotype least square means, and so represent broad-sense genetic correlations roughly comparable to estimates based on family mean values in breeding studies (Via, 1986). To the extent that these broad-sense genetic correlations reflect narrow-sense genetic correlations, they indicate that selection cannot act independently on these three traits. A change in fitness *via* increased allocation to one form of reproduction will be balanced by reduced allocation, and hence presumably reduced fitness, *via* the other two modes of reproduction. In this scenario, selection would result in evolutionary change if fitness advantages due to increased allocation to one mode of reproduction more than offset fitness costs due to decreased allocation to the other two modes.

The selective pressures acting on life-history traits in *B. schlosseri* are not completely understood. Overgrowth by colonies of the con-familial *Botrylloides diegense* has been suggested to select against semelparous, high-egg-production genotypes of *B. schlosseri* in the Eel Pond at Woods Hole (Grosberg, 1982, 1988), and this introduced spatial competitor has also been present in the Damariscotta River since the late 1970s (Yund and Feldgarden, 1992). Selection *via* fertilization processes may also be important, especially in favoring different production levels of male and female gametes (Levitan, 1995; Levitan and Petersen, 1995). For example, ecological situations in which males compete for fertilizations (Yund and McCartney, 1994) or levels of egg fertilization are sperm limited (Levitan, 1995) could both result in selection for increased production of sperm (Yund, 1997). The relaxation of those conditions would in turn favor increased allocation to egg production due to tradeoffs in allocation.

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